CAUSES UNDERLYING THE LOWERING OF THE NATURAL RESISTANCE OF IRRADIATED ANIMALS TO LIVE BRUCELLOSIS VACCINE

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[Following is the translation of an article by Z. V. Shevtsova, Gamaleya Institute of Epidemiology and Microbiology, AMN USSR, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) 1964, No 4, pages 100--105. It was submitted on 29 May 1962. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

During a study of the influence of irradiation on immunogenesis (1959, 1960), we noted that in irradiated animals (guinea pigs, white rats) the natural resistance to a vaccine strain of brucella was lowered. Many investigators observed a lowering in the resistance of irradiated animals to other species of microbes. Various opinions exist relative to the factors which play the leading role in this. Thus, for example, Silverman et al. (1954), while studying the reasons for an increase in the death rate of irradiated animals following their infection with an avirulent plague culture, showed conclusively that an increase in the sensitivity of the animals to the endotoxin of these microbes lies at the base of this phenomenon. Hatch (1955), on the basis of his experimental data, came to the conclusion that endotoxin did not play any role in the raising of lethality among irradiated animals following the administration of Proteus morganii to them. Many investigators observed an intensified multiplication of microbes in an irradiated organism as a result of an impairment of the antimicrobial function. Kiselev et al. (1958) showed that following the administration of the same microorganism the antimicrobial function was disturbed 8--10 times more strongly than the antitoxic function. In addition to this, these same authors (1962) obtained data that following irradiation the antiendotoxic function of the organism suffers more than the antiexotoxic function. Apparently, in each individual case the greatest importance is acquired by the impairment of various functions depending on the properties of the microorganism.

The mission of the present investigation was the study of the problem concerning the factors which play the leading role in the lowering of resistance which we observed in irradiated animals to a vaccine strain of brucella.

The investigations were carried out in guinea pigs (weight 280--300 grams). The animals were irradiated on a twin RUM-3 device with a uniform field 30-40cm, at a voltage of 180 kV, strength of current 15mA, filters 0.5 mm Cu and 1 mm Al; the dosage rate of air on the entire field subjected to irradiation equaled 42 r/ 1 min. The dose of irradiation comprised 200 r and caused the death of 20--30% of the animals used in the test (30 days of observation).

On the 10th day following irradiation a live vaccine culture of Br.

abortus No 19-BA in a quantity of 1 billion microbial cells (based on the optical standard of turbidity) in 1 ml of physiological solution was administered to the animals subcutaneously in the right inguinal area. For a comparison we used a heat killed vaccine culture (heated for an hour at 60°) and the Boivin type antigen from a vaccine culture of brucella.

In the tests on 65 guinea pigs and 40 white rats a study was made of the intensity of seeding of the organs by brucella in the early stage of the vaccination process, that is, during the period in which the maximum death rate of the animals was observed. In a comparison of the number of brucella colonies which grew on agar and their seeding index in organs, no significant differences were observed between the irradiated and nonirradiated animals, (table 1). Analogous data were obtained when studying the early stage of the vaccination process in rats which had been irradiated in doses of 500 r (LD 40/30).

The heat killed vaccine culture of brucella also caused an increase in the death rate of irradiated animals, but to a lesser degree than the live culture (table 2), that is, the products of the death of the microbial cell played a specific role in the phenomenon observed.

As is known, brucella contain an endotoxin which is fixed to the cell. It may be extracted from them by means of extraction with trichloroacetic acid according to the method of Boivin (Lisbonne and Monnier, 1936; Vershilova, 1938, 1940, 1941; Dubrovskaya, 1954).

The antigenic complex which we obtained from brucella of a vaccine strain Br. abortus No 19-BA was nontoxic for guinea pigs following the subcutaneous administration of 0.5--5 ml, but caused the death of white mice (weight 14--16 grams) in the course of 24 hours following the intraperitoneal administration of 1 lethal dose, which equaled 0.75 ml or 0.25 mg. Two lethal doses for mice were administered to guinea pigs, in the first two tests -- 1.5 ml of liquid antigen, in the last one - 0.5 mg of dried. Following the administration of the Boivin type antigen the death of irradiated pigs increased by a still greater degree than following the administration of killed and live brucellosis vaccine (table 3).

As should be expected, the administration of a physiological solution to irradiated animals did not cause any unfavorable effects.

The live or killed vaccine culture of brucella, and also the Boivin type antigen obtained from it, did not cause the death of nonirradiated animals (table 4). Analogous results were obtained when using dry endotoxin (table 5).

Thus, in an irradiated organism there was an increase of sensitivity to live and heat killed brucellosis vaccine, and also to brucella endotoxin. But in order to prove experimentally what role the endotoxin plays in the increased death which is observed following the administration of a live culture, it would be necessary to introduce it into the organism in an amount which is equivalent to that which is released following the administration of 1 billion microbial cells. It is not feasible to do this. We can calculate

what amount of endotoxin is obtained from 1 billion microbes in vitro (0.03 mg), but we do not know how much of it is released in vivo following the administration of the corresponding number of microbial cells. It is unknown how the number of microbes increased in the organism and how many of them were destroyed with the subsequent release of endotoxin. In connection with this, the role of endotoxin in the phenomenon observed may be proven only indirectly, by a comparison of results obtained.

It was shown in our experiments that there was essentially no difference in the intensity of seeding of irradiated and nonirradiated animals by the brucella vaccine culture.

Similar data were also obtained by Pushkarenko and Ivanov (1958) following administration of a virulent culture of brucella. A study of the cultures which were isolated from irradiated animals testified that the cause of increased death could not be an increase in the virulence of the brucella of the vaccine strain (the results of this investigation will be published in a separate report). Along with this, we obtained conclusive data which testified that in the increase of death a specific role was played by the products of the destruction of brucella and that in an irradiated organism there was a significant increase in sensitivity to brucellosis endotoxin. The following fact may be considered as indirect proof of the role of endotoxin: The administration of a small amount of a virulent culture of brucella (50 microbial cells) did not have an influence on the death rate of animals during the height of radiation sickness (Pushkarenko and Ivanov). As is known, death following the administration of small doses of microbes is specified by sensitivity to infection, and of large doses -- to intoxication.

On the basis of what has been said we consider that their increased sensitivity to brucella endotoxin plays the deciding role in the increased death rate of irradiated animals following the administration of live brucellosis vaccine.

Having observed an increase in the sensitivity of irradiated animals to the endotoxin of avirulent plague microbes, Silverman et al. propose that under the influence of irradiation there is an increase in the sensitivity of the host cells which come in contact with the endotoxin. The authors consider that the cause of increased sensitivity to endotoxin will remain unclear as long as the mechanism of its action is unknown. We would like to take exception to the authors, that in addition to the true sensitivity of host cells, which are the point of application of the endotoxin, there may be significance in other factors, primarily a disruption of the natural process of detoxification. This same opinion is held by Kiselev et al. (1962), who consider that in an irradiated organism the mechanisms for the detoxication of microbial toxins are impaired. In contrast to exotoxins, which are destroyed mainly by humoral mechanisms, the disintegration of endotoxins takes place mainly with the help of cellular factors, in particular cells of the reticulo-endothelial system. The authors showed that in irradiated animals the capability of the splenic tissue to destroy endotoxins is impaired. However, the problem concerning the mechanism of rendering

endotoxins harmless under normal conditions and following irradiation requires a further comprehensive study. Here it is necessary to take into consideration the role of the hormone system. It is interesting that in adrenalectomized animals, just as in irradiated ones, there is an increase in sensitivity to endotoxins (Ioffe, 1962).

Conclusions

1. An increase in the death of irradiated laboratory animals is observed following the administration of live or heat killed vaccine cultures of brucella, and also by the administration of the endotoxin which is extracted from them.

2. The intensity of seeding of organs by the brucella vaccine strain was not intensified and its residual virulence was not increased.

3. In the lowering of the resistance of irradiated animals to the vaccine strain, Br. abortus No 19-BA, a leading role resides in the increased sensitivity to the brucella endotoxin.

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Table 1

Recovery of a brucella vaccine culture from the organism of guinea pigs, irradiated with 200 r.

Vaccination in 10 days following irradiation									Vaccination without irradiation								
Time passed following vaccination (in hours)	Intensity of growth of brucella (no. of colonies) & seedings from organs and tissues							Seeding index (in %)	Time passed following vaccination (in hours)	Intensity of growth of brucella (no. of colonies) & seedings from organs and tissues							Seeding index (in %)
	Regional lymph nodes	Distal lymph nodes	Liver	Spleen	Urine	Blood	Marrow			Regional lymph nodes	Distal lymph nodes	Liver	Spleen	Urine	Blood	Marrow	
3	over 80	1-10	10-30	over 80	1-10	10-30	30-80	77.2	3	over 80	1-10	1-10	over 80	10-30	30-80	1-10	94.4
6	" 80	1-10	10-30	" 80	1-10	10-30	1-10	83.3	6	" 80	1-10	10-30	" 80	1-10	10-30	1-10	83.3
12	" 80	1-10	10-30	" 80	1-10	10-30	10-30	94.4	12	" 80	1-10	10-30	" 80	1-10	1-10	1-10	94.4
24	30-80	1-10	1-10	10-30	-	1-10	1-10	44.4	24	30-80	-	1-10	" 80	1-10	1-10	1-10	66.6
48	10-30	1-10	1-10	1-10	1-10	-	1-10	55.5	48	10-30	1-10	1-10	10-30	-	-	1-10	66.6
72	30-80	1-10	1-10	10-30	1-10	-	1-10	55.5	72	10-30	-	1-10	10-30	-	-	1-10	44.4
24x5	30-80	1-10	1-10	10-30	-	1-10	1-10	55.5	24x5	30-80	1-10	-	1-10	-	-	1-10	55.5
24x15	30-80	1-10	1-10	1-10	-	-	1-10	50	24x15	30-80	1-10	1-10	1-10	-	-	1-10	50
24x30	10-30	1-10	1-10	1-10	-	-	1-10	33.3	24x30	10-30	1-10	-	-	-	-	1-10	22.2

Table 2

Influence of live and killed vaccine cultures of brucella on irradiated guinea pigs

Preparation administered	No. of animals	Number which died							
		Total	on which day following irradiation						
			10th	11th	12th	13th	14th	17th	20th	27th
-	22	6 (27%)	1	1	2	2	-	-	-	-
Killed culture 19-BA	22	11 (50%)	2	2	3	2	1	-	-	1
Live culture 19-BA	22	15 (68%)	2	1	4	5	-	2	1	-
Physiological solution	22	5 (23%)	1	1	2	1	-	-	-	-

Table 3

Influence of various preparations of brucella on irradiated guinea pigs

Preparation administered	No. of animals	Number which died										
		Total	on which day following irradiation									
			2nd	11th	12th	13th	14th	15th	16th	17th	21st	23rd	24th
-	27	6 (22%)	1		1	2	1	-		1			
Killed culture 19-BA	30	10 (33%)			2	1	3	2	1			1	
Live culture 19-BA	52	23 (44%)			2	2	6	3		4	2	2	2
Boivin type antigen	32	22 (69%)	3	8	4	4		1	2				
Physiological solution	24	4 (17%)	1	2			1						

Table 4

Death of irradiated guinea pigs following the administration of various preparations of brucella

Group	Preparation administered	No. of animals	Number which died								
			Total	on which day following irradiation							
				9th	10th	11th	12th	13th	14th	15th	16th	18th
Irradiated	-	16	4 (20%)	1	1	1			1			
	Killed brucella vaccine	16	7 (44%)				1	1		1	3	1
	Live brucella vaccine	16	11 (60%)				2	4	2	2	1	
	Boivin type antigen	16	14 (87%)	1	1		6	4	1		1	
Nonirradiated	Killed brucella vaccine	16	0									
	Live brucella vaccine	16	0									
	Boivin type antigen	16	0									

Table 5

Influence of live brucellosis vaccine and the Boivin type antigen on the death of irradiated guinea pigs

Group	Preparation administered	No. of animals	Number which died							
			Total	on which day following irradiation						
				Before 10	10th	11th	12th	13th	14th	15th	21st
Irradiated	-	20	6 (30%)	2	1	2	-	1	-	-	-
	Live brucella vaccine	20	13 (65%)	3	1	5	2	2	-	-	-
	Boivin type antigen	20	14 (70%)	2	2	4	5	-	-	-	1
Nonirradiated	Live brucella vaccine	10	0								
	Boivin type antigen	10	0								